Introduction

Wetlands are vital water bodies, as crucial in a natural ecosystem as a kidney in a human body. As defined by the Ramsar Convention on Wetlands, wetlands are “areas of marsh, fen, peat land or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters” (Article 1.1 of the Convention text). Approximately 6% of Earth’s land surface, which equals about 2 billion acres, is covered by wetlands. Wetlands are one of the most productive ecosystems, comparable to tropical evergreen forests in the biosphere and play a significant role in the ecological sustainability of a region.

Wetlands being one of the most biologically productive natural ecosystems are vital for the survival of diverse flora and fauna, including many threatened and endangered species by providing shelter, food, etc., and forming a part of the complex food-web. Wetlands are a critical feature of the global landscape because of their unique role in regulating global biogeochemical cycles. Biogeochemical processes occurring in wetland ecosystems are also important in global biogeochemical cycles, including global warming, carbon sequestration, and water quality besides acting as sources of carbon dioxide, methane, and nitrous oxide. Microbial communities are pivotal to many biogeochemical processes functioning in wetlands.

Heterotrophic bacteria performing the processes of organic matter decomposition are the most numerous group of microorganisms in aquatic ecosystems (Karner *et al*. 1992). As there are differences between various types of water bodies in the composition and content of organic matter, the intensity of biodegradation by bacterial microflora differs as a function of the biochemical activity of the dominant physiological groups. Isolated bacterial groups have been intensively investigated, little attention has been given to their relation to the aquatic environment, and little is known about the numerous aerobic heterotrophic bacteria occurring in un-polluted environments.

The management and exploitation of microbial diversity has an important role in sustainable development with the industrial and commercial application of microbial diversity worth millions of pounds. However, despite the obvious economic value of microbial diversity,

knowledge on microbial diversity is rather poor. Vembanadu lake is an internationally important Ramsar site and expected to support good microbial diversity. The present study is an attempt towards estimating, characterising and inventorying the culturable heterotrophic bacteria in the water and sediment at Kumarakom region of Vembanadu lake.

Study area

The study has been conducted in the Kumarakom region (9°37'57"-9°38'21"N Latitude, 76°25'11"-76°25'06"E Longitude) of Vembanadu-Kol wetland of Kerala, along the southwest coast of India. Vembanadu lake, which is connected to Arabian sea through Cochin estuary, is the largest brackish, tropical wetland ecosystem, which is of extraordinary importance for its hydrological function, biodiversity and rich fishery resources. Kumarakom is situated on the eastern banks of Vembanadu lake. Though the biodiversity of this wetland at macrolevel is well documented there are virtually no reports available on the microbial diversity of the Kumarakom wetland ecosystem.

Objectives

1. Collection and analysis of water and sediment samples from Kumarakom region of Vembanadu lake for their physico-chemical and bacterial community analysis.
2. Estimation and isolation of culturable aerobic heterotrophic bacteria and actinomycetes from water and sediment samples of Kumarakom region of Vembanadu lake through classical microbiological tools.
3. Characterisation of culturable heterotrophic bacteria at species level
4. Bioprospecting of potential bacterial genera for hydrolytic enzymes and Actinomycetes for antibacterial substances.
5. Molecular characterization of potential strains of Actinomycetes using 16s rRNA based identity.

6. Inventorization and storage of the characterized heterotrophic bacteria for further research.

Methodology

Analysis of physico-chemical parameters of water and sediment samples: Physico-chemical parameters of water and sediment samples were analyzed as per standard methods prescribed in APHA. Standard methods were employed for the assessment of nutrients in sediment.

Quantification of culturable aerobic heterotrophic bacteria from sediment and water samples: Water and sediment samples were processed according to the standard procedures and methods. Appropriate dilutions were inoculated in duplicate, by spread plate/pour plate method, on various media to enumerate the bacterial count. Tryptone Soya Agar (TSA) and Nutrient Agar (NA) were used for the enumeration of total aerobic heterotrophic bacteria. TSA/NA media used for spread plate and pour plate was prepared with distilled water and also by using filtered lake water. Inoculated plates were incubated at ambient temperature and at 37ºC. After incubation the colony was enumerated and was recorded as colony forming units in water (cfu/mL) and sediments (cfu/g).

Identification of the bacterial isolates: Chosen bacterial isolates were subcultured, purified and identified using morphological, physiological and biochemical tests as per Bergey's manual of determinative bacteriology, 8th edition (Buchanan and Gibbons, 1974) and Bergey's manual of determinative bacterology, 9th edition (Holt *et al*., 2000).

Isolation and evaluation of antibacterial activity of actinomycetes: Actinomycetes were isolated from sediment samples by using Kuster's agar and their antibacterial activity was determined by well diffusion method using agar wells in Glycerol-Yeast Extract Agar (Waksman, 1961). DNA was isolated from most bioactive actinomycete isolates as per Liu *et al*. (2000) and was amplified and sequenced. The sequences obtained were compared for similarity level with the reference species of microorganisms contained in genomic database banks, using 'NCBI Blast' (http://www.ncbi.nlm.nih.gov/) and phylogenetically assigned according to their

best matches to sequences in the NCBI database/ RDP-ribosomal database project: (http://rdp.cme. msu.edu/).

Results

Analysis of the physico-chemical parametes of the water and sediment samples from Kumarakom lake region revealed both spatial and seasonal variation. Sediment pH showed significant positive correlation with exchangeable potassium in the sediment. Water samples recorded maximum value of salinity during pre-monsoon (4.2‰) season and minimum during the monsoon season (0.08‰). Seasonal variation of sodium and potassium are statistically significant. Textural analysis revealed the predominance of sandy clay loam in the Kumarakom lake sediments.

The average number of culturable bacteria in the present study sites ranged from $1.90x10^5$ to $9.70x10^5$cfu/gm in the sediment samples and from $2.50x10^3$ to $6.20x10^3$cfu/mL in water samples. Significant difference in the bacterial count was observed in spread plate and pour plate ($p<0.001$) method. On an average higher bacterial load was obtained in the spread plates of TSA/NA prepared with lake water which was incubated at room temperature.

A total of 15 bacterial genera were identified in sediment and 17 genera in water samples. The genera *Acinectobacter* and *Lactobacillus* were identified from water samples only. Thirty Gram positive bacterial species were identified in water samples, which were distributed in 5 genera, such as *Bacillus*, *Listeria*, *Kurthia*, *Carnobacterium* and *Staphylococci*. Thirty seven Gram positive bacterial species were identified from sediment of the study area, which were distributed in 5 genera, such as *Bacillus*, *Listeria*, *Kurthia*, *Carnobacterium* and *Staphylococci*. While 40 species of Gram negative bacteria were identified in water of Kumarakom region of Vembanadu lake, only 11 species of Gram negative bacteria were identified in sediment.

About 73% of sediment derived bacterial isolates and 41% of bacterial isolates from water were identified as *Bacillus*. Twenty nine *Bacillus* species were identified in the sediment samples and 20 species from water. *Bacillus subtilis* and *Bacillus cereus* was found to be the most dominant *Bacillus* species both in water and sediment, based on their percentage of incidence.

Nitrate reductase was detected in 40 and 46% of *Bacillus* isolates from water and sediment respectively. While 83% of *Bacillus* isolates from sediment have protease activity; it was only 78% those in the isolates from water. All the *Bacillus* isolates from water samples revealed amylase activity, while only 75% of *Bacillus* isolates from sediment samples showed amylase activity. Tyrosinase was detected in 15% of *Bacillus* isolates from sediment and 13% of *Bacillus* isolates from water. Twenty two percentage of *Bacillus* isolates from sediment samples were able to survive up to 55ºC. About 15% of *Bacillus* isolates from water samples of Kumarakom lake were also survived up to 55ºC.

*L. ivan*ovii, *L. murrayii* and *L. grayi* were identified from water samples. Species of *L. seeligeri* and *L. welshimeri* detected in sediment samples. *K. zopfii, C. gallinarum, S. aureus* and *S. epidermidis* were identified from water and sediment samples. β-haemolytic activity was detected in 40% of *Listeria* isolates from water and 50% of *Listeria* isolates from sediment. Coagulase activity was detected in 50% of *Staphylococci* isolates from water and 67% of isolates from sediment. Phosphatase activity was detected in 40% of *Kurthia* isolates from sediment.

Four species of Enterobacteriaceae were identified in sediment of Kumarakom lake and 11 species from water. While *Proteus vulgaris* was the dominant Enterobacteriaceae in sediment, *Enterobacter cloacae* was the dominant one in water. Three species of *Vibrio* were identified in sediment of Kumarakom lake and 17 species of *Vibrio* were identified from water samples. *V. coralliilyticus* was identified from water samples collected from all five stations. More diverse *Vibrio* species were identified in water during the month of June. Various species of *Aeromonas* identified in this study were isolated from water samples collected during warmer times of the year. Five species of *Aeromonas* and *Alcaligenes* and two species of *Pseudomonas* were identified from water in the study area. Presence of 4 species of *Cytophaga* was also encountered in sediment.

A total of 27 diverse actinomycetes were isolated from the sediment samples from Kumarkom region of Vembanadu lake. They were identified, based on their morphological, physiological and 16s rRNA gene sequences, as *Actinobispora*, *Actinosynnema*, *Actinokineospora*, *Catellospora*, *Rhodococcus*, *Kibdelosporangium*, *Micromonospora*, *Nocardiopsis* sp. BF11, *Streptomyces* sp. FXJ3.007, *Streptomyces costaricanus* WZ162, *Streptoalloteichus*,

Saccharopolyspora, *Streptosporangium*, *Thermoactinomyces* and atypical *N. asteroids*. About 56% of actinomycete isolates decomposed hypoxanthine and tyrosine. The productivity of actinomycete strains as antibiotic producers remain unique amongst the microbial world. It was observed that, more than 90% of actinomycetes from lake sediments suppressed in different degrees the growth of the test pathogens. Thirty seven percent of actinomycete isolates from Kumarakom lake sediments were active against enteropathogenic *E. coli*. While 30% of sediment derived actinomycetes showed antibacterial activity against enterotoxigenic *E. coli*, about 33% showed antagonism towards *V. cholerae*.

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